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Effect of Low-Intensity Laser Irradiation (660 nm) on a Radiation-Impaired Wound-Healing Model in Murine Skin

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Background and Objective: **The use of low-intensity laser therapy (LILT) as a therapeutic modality has become popular in a variety of clinical applications including the promotion of wound repair. Although the clinical evidence base for such application remains sparse, recent studies have demonstrated a number of quantifiable photobiological effects associated with such therapy. In the present study, the effect of laser irradiation at various radiant exposures on a radiation-impaired wound-healing model in murine skin was investigated.**
Study Design/Materials and Methods: **The study included two phases; in phase one, male Balb/c mice (n = 36; age-matched at 10 weeks) were randomly allocated to three experimental groups (n = 12, each group). In all groups, a well-defined area on the dorsum was exposed to 20 Gy x-rays. Seventy-two hours postirradiation, all mice were anaesthetised and a 7 × 7 mm area wound was made on the dorsum. All wounds were videotaped alongside a marker scale (three times weekly) until closure was complete. In groups 2 and 3, mice were treated with laser irradiation (0.5 and 1.5 J/cm^2, respectively) three times weekly by using a 660-nm GaAlAs laser unit (5 kHz; 15 mW; Omega Laser Systems, London, UK). Wound areas were then calculated by using an image analysis system (Fenestra 2.1), and results were analyzed by using repeated measures and one-factor analysis of variance statistical tests. In phase two, two experimental groups were included (n = 12 each group); the protocol was identical to that described for phase 1; however, mice in group 2 were treated with a radiant exposure of 4 J/cm^2.**
Results: **Results from this investigation demonstrated that treatment with 0.5, 1.5. and 4 J/cm^2 had no beneficial effect on the rate of wound closure ($P > 0.05$).**
Conclusion: **These findings provide little evidence of the putative stimulatory effects of LILT in vivo at the parameters investigated. Lasers Surg. Med. 26:41–47, 2000.** **© 2000 Wiley-Liss, Inc.**

Key words: full-thickness skin excision; laser therapy

INTRODUCTION

Since the effects of laser photobiostimulation [*sic*] were first investigated by Professor Endre Mester in a murine model in 1971, Low-intensity laser therapy (LILT) has gained wide-

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spread acceptance within the physiotherapy profession for the treatment of pain and the promotion of wound healing [1]. Clinically, laser therapy is used to treat a variety of aetiology, including arthritic conditions and soft-tissue injuries. However, despite the extensive availability of lasers within the clinical field, there is still uncertainty concerning the most suitable treatment parameters [1]. Although a substantial amount of research has been conducted on the biological and physiological effects of laser therapy [2–4], further work is required to improve our understanding of the mechanisms of action of this modality and its potential for wound healing.

Previous studies have evaluated the efficacy of laser irradiation in a variety of animals, healthy humans, and subjects with varying pathologic conditions and lesions; these have shown that, depending on the dose, duration, wavelength, and type of tissue, the effect of irradiation may be stimulating, inhibiting, or even damaging [5]. In the clinical setting, LILT has been reported as effective in the treatment of chronic venous ulceration [6], crural ulcers [7], soft-tissue lesions, and for the stimulation of both acute and chronic wound healing [8].

The majority of authors have reported that laser biostimulation [*sic*] of wound healing most often occurs during the proliferative phase [9–11]. It is thought that such effects occur due to the enhancement of cell metabolic processes as a result of laser therapy, thereby affecting the electrophysiological properties of the tissue [12]. Laser irradiation has been shown to affect most of the cell types involved in wound healing: in two recent studies, laser irradiation was reported to activate fibroblasts [13,14]. Young and Dyson [15] also reported that laser therapy stimulated macrophages and, therefore, is effective during the inflammatory stage of repair; the authors concluded that laser irradiation stimulates macrophages to release chemical mediators, cytokines, and growth factors, which in turn activate the latter stages of wound repair. A recent study has further demonstrated that irradiation by using an Argon laser (630 nm and 660 nm) caused the release of transforming growth factor and platelet-derived growth factor from fibroblasts [16]; these authors also measured cytokine production by enyzme-linked immunosorbent assay (ELISA) and found a significant difference between the laser irradiated and control groups. Another study by Bolton et al. [10] indicated an increase in the proliferation of macrophage-like cells by using a wavelength of 660 nm and a radiant exposure of 7.2 J/cm^2. Collagen synthesis has also been shown to be sensitive to laser irradiation [17]. This finding is supported by Mester and Jaszsagi-Nagy [18], who observed that collagen synthesis following pulsed ruby laser irradiation of 4 J/cm^2 exceeded the control group by 30–50%. However, it should be noted that, although these findings are generally positive, results for some cell types have tended to be more variable. Laser irradiation of lymphocytes has typically shown inhibitory effects on cellular proliferation [19]; other groups have also failed to find any significant laser-mediated effect on fibroblasts [20].

In animal studies, encouraging results have been reported in small loose-skinned animals such as rats and mice [3,21,22]; however, results observed clinically remain less convincing [23,24]. Mice are often used for such research because they are the most practical model of wound repair, being inexpensive, available in large numbers, and having a low death risk on anesthesia [1]. These factors, together with ease of handling and the fact that healing occurs within 2–3 weeks of wounding, make the use of these animals ideal for such research [25,26]. Additionally, to stimulate pathologic wounds similar to those found clinically (e.g., in elderly and diabetic patients), an impaired wound-healing model is necessary. Such an impaired wound-healing model can be induced through x-ray irradiation; although its effect has been found to be dose-dependent [27], it has been reported recently that x-ray irradiation of a single 20-Gy dose to the skin delays wound healing by 7 days [28–30].

In a previous study [28], this group has demonstrated that low-intensity monochromatic irradiation (890 nm) at a variety of energy densities had no beneficial effect on the rate of wound closure; indeed, inhibitory effects were observed at the highest energy density used. For the current study, a red light source was investigated (660 nm) to establish whether putative stimulatory effects may be wavelength dependent, because most positive studies to date have been with the use of visible red light sources [31–33]. Furthermore, a survey of clinical practice has indicated red light sources to be more popular in the treatment of wounds [34]. To this end, the aim of the current study was to investigate the effect of GaAlAs (660 nm) laser irradiation on the rate of wound closure of a radiation-impaired wound in murine skin and to determine whether such an effect was dose dependent.

MATERIALS AND METHODS

Phase 1

The current investigation included male Balb/c mice, age-matched at 10 weeks old (n = 36; mean weight = 26.88 g); all were supplied with food and water ad libitum, and housed individually to prevent cross-tampering with wounds. Animals were then randomly assigned to one of three experimental groups (n = 12, each group): Group 1, x-ray–irradiated control; group 2, LILT at 0.5 J/cm^2; group 3, LILT at 1.5 J/cm^2.

The hair on the dorsum of the mice was shaved; all mice were then placed individually into custom-made lead jigs, which allowed a 4 cm^2 area of dorsal skin to be exposed. The area was marked with indelible ink, and the mice were exposed to 20 Gy x-ray irradiation by using a Siemens Stabilipan x-ray machine (Siemens Ag. Medical Group, Erlagen, Germany). Seventy-two hours after x-ray irradiation, hair on the dorsal surface was reshaved (where required) and the skin was cleaned with 70% alcohol. Mice in all groups were then anaesthetised by inhalation of isoflurane anaesthetic (Abbot Laboratories, Ltd., Essex, UK) and a 7 × 7 mm full-thickness skin excision was made within the area previously exposed to x-rays.

In groups 2 and 3, mice were subsequently irradiated at radiant exposures of 0.5 and 1.5 J/cm^2, respectively; this was delivered by using a modulated output single diode (GaAlAs; Omega Laser Systems, London, UK). The physical parameters of this unit were measured as wavelength: 660 nm; maximum rated power output, 15 mW; area of irradiation, 0.336 cm^2; irradiance, 0.045 W/cm^2; pulsed (modulated) at 5 kHz. Power output was checked at the beginning of each day; irradiation times for radiant exposures of 0.5 and 1.5 J/cm^2 (14 and 42 seconds, respectively) were calculated by using the equation:

$$\text{Time (seconds)} = \frac{\text{Energy density (J/cm}^2\text{)}}{\text{Irradiance (W/cm}^2\text{)}}$$

During irradiation, the laser probe was held with the tip just in contact with the dorsal surface of the wound; all animals were anaesthetised by inhalation of isoflurane anesthetic before irradiation. Treatment was given three times weekly until complete wound closure. Group 1, the control group, received no treatment.

All wounds were videotaped before laser irradiation alongside a marker scale three times weekly thereafter until closure was complete. Wound areas were then calculated by using an image analysis system (Fenestra v2.1; Kinetic Imaging, Ltd., Liverpool, UK). To allow for any variation between the initial size of the wound on each animal, data were expressed as a fraction of the initial wound area for each mouse. Results were analyzed in a blind manner by using repeated measures analysis of variance (ANOVA), together with one-factor ANOVA and post hoc Fisher's exact tests where appropriate.

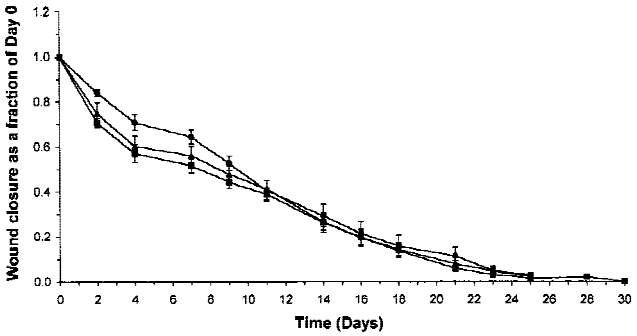

Fig. 1. Wound closure as a fraction of day 0 for x-ray–irradiated control and low-intensity laser therapy treatment group at 0.5 J/cm^2 and 1.5 J/cm^2 (points represent means ±SEM; n = 12 for all groups). Squares, group 1, x-ray–irradiated control; triangles, group 2, x-ray–irradiated + LILT at 0.5 J/cm^2. Circles, group 3, x-ray–irradiated + LILT at 1.5 J/cm^2.

Phase 2

The experimental procedures used in this phase were identical to those described in phase 1. Animals were again age-matched at 10 weeks old (n = 24; mean weight = 27.01 g) and randomly assigned to one of two experimental groups (n = 12, each group); group 1, x-ray–irradiated control; group 2, LILT at 4 J/cm^2. The irradiation time was calculated as 112 seconds for group 2.

RESULTS

Findings from the current investigation demonstrated that the overall rate of wound healing in this murine model was not accelerated following laser irradiation by using a 660-nm GaAlAs laser at radiant exposures of 0.5, 1.5, and 4 J/cm^2.

Calculated wound areas as a fraction of day 0 for phase 1 are presented in Figure 1; each data point represents the means ± SEM. Statistical analysis of data in phase one by using analysis of variance showed that treatment with 0.5 and 1.5

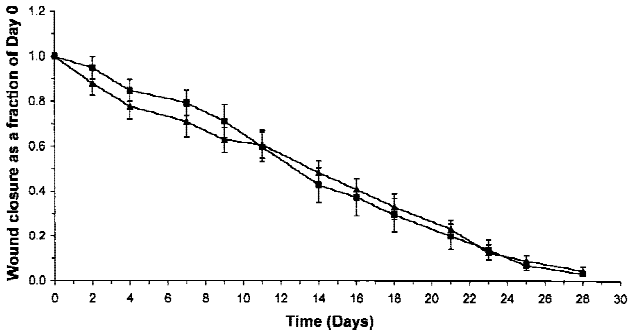

Fig. 2. Wound closure as a fraction of day 0 for x-ray–irradiated control and low-intensity laser therapy (LILT) treatment group at 4 J/cm^2 (Points represent means ±SEM; n = 12 for all groups). Squares, group 1, x-ray–irradiated control; triangles, group 2, x-ray–irradiated + LILT at 4 J/cm^2.

J/cm^2 had no effect on the rate of wound closure in this animal model ($P = 0.27$). It can be clearly seen that there was little difference between the x-ray–irradiated control and the LILT treatment group at 0.5 J/cm^2 throughout the duration of the experiment. In contrast, the 1.5 J/cm^2 dose appeared to inhibit wound healing between days 2 and 7; however, these values were not shown to be significantly different. All groups achieved complete wound closure by day 30.

In phase 2 of the investigation, no differences were observed between the x-ray–irradiated control and the 4.0 J/cm^2 treatment group at any time point. Figure 2 shows wound area as a fraction of day 0 plotted against time; again each data point represents the mean ± SEM. The control and treated group are essentially indistinguishable, with all wounds being fully closed by day 30 post-wounding. Statistical analysis demonstrated no differences between groups ($P = 0.56$). To summarise, treatment with LILT at 0.5, 1.5, and 4 J/cm^2 had no effect on the rate of wound closure in this model when compared with the x-ray–irradiated control group.

DISCUSSION

The aim of the current study was to investigate the effect of 660-nm GaAlAs laser irradiation on the rate of wound closure in a radiation-impaired wound in murine skin, and to determine whether any such effect was dose dependent. Findings from the current study demonstrated that laser irradiation at 0.5, 1.5, and 4 J/cm^2 had no beneficial effect on the rate of wound closure in this murine model. These findings are in agreement with those demonstrated previously by Lowe et al. [28]; these authors reported that laser irradiation at similar radiant exposures by using an 890-nm laser also had no beneficial effect on wound closure in the same model.

Results obtained from previous studies have demonstrated that prior irradiation with x-ray doses of 20 Gy caused a significant delay in the rate of wound healing by day 7 when compared with the non–x-ray–irradiated control [18,30]; Bernstein et al. [29] also reported similar findings. The impaired wound-healing model used in the present study showed a significantly extended proliferative phase during the wound-healing process, whereas at the inflammatory and remodelling phases this delay was not found to be significant. Such an effect may occur as a result of x-ray irradiation, because there is a delayed progression of the epidermal cells in the cell cycle, thus leading to a reduction in fibroblast production in the granulation bed [35].

Interpreting laboratory results on the effects of LILT from research to date is difficult due to the diversity of animal models used as well as variation in (and/or lack of detail on) parameters [8]. In addition, many authors that used similar laser units and protocols have reported conflicting findings. However, the results of the current study support the recent findings of Allendorf et al. [36] in which laser irradiation by using a He-Ne (632.8 nm) laser at radiant exposures of 1, 2 and 4 J/cm^2 produced no measurable benefit on wound healing in healthy rats. Likewise, a study by Hall et al. [4] reported no effects on the rate of wound closure of open skin wounds in rats when using laser irradiation at radiant exposures between 0.4 and 4 J/cm^2. However, previous studies have shown an accelerated rate of wound healing following irradiation again at energy densities of between 1 and 4 J/cm^2 [3,37,38]. In addition, earlier work by Kana et al. [31] demonstrated that laser irradiation by using an Argon laser increased collagen production in wounds in rats but did not accelerate the overall rate of wound healing. Again in contrast to the current study, Lee and Kim [39] reported accelerated wound closure by using a GaAs laser (904 nm), at a radiant exposure of 76.4 mJ/cm^2, on an infected open wound in murine skin. Likewise, many other studies have reported that laser irradiation was found to increase the rate of wound closure in rats [31] and mice [40]. Other reports have shown He-Ne laser irradiation also improved wound tensile strength in rats [41] and mice [42].

As previously outlined, LILT has been re-

ported to stimulate the proliferative phase of wound healing [3,10,31,43]; other authors have reported stimulatory effects in the inflammatory phase [13]. One proposed mechanism by which LILT stimulates the wound-healing process is by the absorption of light energy by the mitochondria, which increases cell energy and stimulates the release of chemical mediators [3,25,44]. It would appear that such a mechanism did not occur in the current study because of the lack of a stimulatory effect. This may be due to insufficient light energy reaching the cells. Allendorf et al. [36] have suggested laser light penetrations of tissue and eschar debridement are concerns within wound healing. Wounds that are not debrided such as the wounds in the current study may not allow the maximal amount of light to reach the tissue. This possibility is supported by Surinchak et al. [41], who have ascertained that debrided wounds contract faster than those left intact, irrespective of laser treatment. In addition, Allendorf et al. [36] also suggested that anesthesia produces a systemic effect that may retard or augment the wound-healing process. These authors determined that if the effects are profound enough, they might obscure or overwhelm the effects of laser treatment; thus, it would be necessary to exclude any adverse effects of anesthesia before drawing any conclusions about the effect of LILT.

Several studies have shown isolated improvements in the various stages of wound healing, but none as yet have shown any beneficial effects on the overall rate of wound healing [45]. One explanation for this is that the healing process already proceeds at a near optimal rate; thus, laser cannot increase this rate any further [26,46]. Therefore, it is necessary to use an impaired wound-healing model to determine any stimulatory effects. In the current study, such impairment was achieved by exposing the animals to 20 Gy x-ray radiation prior to wounding. Previous studies showed that x-ray irradiation delays wound healing by day 7 [28]. Although this delay allows more time to investigate the effects of LILT, the irradiation-impaired model cannot be compared directly with the delayed healing observed clinically in pathologic wounds. Other factors that also hinder repair include age, malnutrition, anemia, diabetes mellitus, and infection, and such factors are commonly observed in the clinical setting. In an effort to provide a more clinically relevant wound-healing model, Yu et al. [45] investigated the effects of an argon dye laser (630 nm) on diabetic mice. Findings from that study demonstrated a significant increase in the percentage of wound closure over time by using a radiant exposure of 5 J/cm^2 when compared with the control group. In addition, histologic evaluation showed that laser irradiation improved wound epithelialization, cellular content, granulation tissue formation, and collagen deposition in the laser-treated groups.

Mice are not seen as the best choice for investigators because of their loose skin; wounds in mice heal a great deal by wound contraction rather than reepithelialization, such as occurs in human skin [1]. Therefore, any conclusions drawn from studies on mice cannot necessarily be extrapolated directly to humans, only to guide further research. Future work should investigate the effects of LILT on other animal models, such as tight-skinned animals (e.g., pigs), so that the findings would be more applicable to human wound healing. However, additional problems could occur in these animals due to the increased thickness of pigskin and the fact that the depth of penetration of the laser irradiation will, thus, be decreased. This not withstanding, such investigations may provide an explanation as to the mechanisms of action of these devices. In addition to producing an alternative and more suitable wound-healing model, it may be also beneficial to examine the wounds histologically to detect potential differences between controls and experimental groups at the cellular level. Therefore, future work in this area should also involve characterisation of the wound-healing model following LILT.

Although there is widespread acceptance for the use of LILT in the clinical setting, there is still a lack of scientific evidence and insufficient guidelines in the use of the most effective parameters for laser treatment. Therefore, rigorous trials that use laser units and doses that are continuously modified are required to establish the optimal parameters in both the animal models and in clinical conditions [8]. Delayed wound healing is a clinical problem seen mainly in elderly patients, diabetics, and patients undergoing radiation treatment such as cancer patients. These together with burns patients, and patients with acute and chronic wounds stand to benefit greatly from further research carried out in this field.

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